

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION		1b. RESTRICTIVE MARKINGS	
AD-A216 676		NA	
		3. DISTRIBUTION / AVAILABILITY OF REPORT	
		Distribution Unlimited	
(S)		5. MONITORING ORGANIZATION REPORT NUMBER(S)	
NA		NA	
6a. NAME OF PERFORMING ORGANIZATION	6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION	
University of California, at Los Angeles	NA	Office of Naval Research	
6c. ADDRESS (City, State, and ZIP Code)		7b. ADDRESS (City, State, and ZIP Code)	
Department of Biology UCLA Los Angeles, CA 90024-1606		800 N. Quincy St. Arlington VA 22217-5000	
8a. NAME OF FUNDING / SPONSORING ORGANIZATION	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
Office of Naval Research	ONR	N00014-88-0352	
8c. ADDRESS (City, State, and ZIP Code)		10. SOURCE OF FUNDING NUMBERS	
800N Quincy St. Arlington VA 22217-5000		PROGRAM ELEMENT NO.	PROJECT NO.
		TASK NO.	WORK UNIT ACCESSION NO.
11. TITLE (Include Security Classification)			
(U) Molecular Mechanisms in Cell Wall Assembly and Calcification of Marine Phytoplankton			
12. PERSONAL AUTHOR(S)			
Gonzalez, Elma & Chapman, David J.			
13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
annual	FROM 8/88 TO 8/89	89/12/29	3
16. SUPPLEMENTARY NOTATION			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	Coccolithophorids, calcification, Golgi, membranes, proteins, (Sten)	
19. ABSTRACT (Continue on reverse if necessary and identify by block number)			
<p>This research program involves studies on the molecular and cellular basis for the calcification of the cell walls of coccolithophorids. The optimal conditions for growth and mineralization of <u>Coccolithus pelagicus</u> are being identified. The impact of environmental conditions such as light intensity, pH and mineral nutrition on the extent of coccolithogenesis and on the fidelity of coccolith morphology are being examined by means of light microscopy, and scanning and transmission electron microscopies.</p>			
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT		21. ABSTRACT SECURITY CLASSIFICATION	
<input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS		(U)	
22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL	
M. Marron	202/696-4760	ONR	

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JAN 08 1990
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DATE: 29 December 1989

PROGRESS REPORT ON CONTRACT N00014-88-0352

PRINCIPAL INVESTIGATOR: Elma Gonzalez

CO-PRINCIPAL INVESTIGATOR: David J. Chapman

CONTRACTOR: University of California at Los Angeles

CONTRACT TITLE: Molecular Mechanisms in Cell Wall Assembly and
Calcification of Marine Phytoplankton

START DATE: 1 August 1988

RESEARCH OBJECTIVE: To investigate the molecular and cellular basis for cell wall calcification in coccolithophorids.

PROGRESS (YEAR 1): We have progressed through a tedious year of basic investigations designed to reveal optimal conditions for growth and mineralization of 3 candidate organisms; Coccolithus pelagicus, Hymenomonas carterae, and Pleurochrysis scherffelli. Both synthetic and semi-synthetic growth media have been tested and parameters such as generation times, and the percentage of cells exhibiting calcified coccoliths have been monitored using light and scanning electron microscopy. The influence of environmental factors such as light intensity, aeration, and buffering capacity of the growth medium, on coccolithogenesis have also been examined.

A detailed ultrastructural analysis was carried out on Coccolithus pelagicus to determine the effect of inorganic sulfate depletion on coccolithogenesis. The abundance of mineralized coccoliths and the fidelity of their morphology was monitored by means of scanning electron microscopy. The cultures grown on completely synthetic medium containing inorganic sulphate concentrations ranging from 60 to 30,000 micromolar were also examined by transmission electron microscopy. We observed severe damage to cellular ultrarstructure at the lowest levels of sulfate. However, we did not observe an impact on the normal morphology of coccoliths that could not be explained by the overall derangement of cellular integrity caused by low sulfate. On the basis of these results we tentatively conclude that the templating mechanism underlying morphological fidelity of coccoliths is not sensitive to low sulfate in the growth medium.

We have initiated a preliminary evaluation of techniques for DNA and mRNA isolation from Coccolithus pelagicus. We have successfully translated C. pelagicus mRNA in vitro. These studies will be extended into Year 2.

WORK PLAN (YEAR 2): We have two objectives for Year 2. The first objective is to continue our attempts to isolate and characterize the nucleic acids of C. pelagicus. mRNA will be isolated from cell cultures undergoing extensive coccolithogenesis. After in vitro translation to ascertain the integrity of the isolated mRNA we will undertake construction of a cDNA library. The second objective is to characterize the membrane polypeptides of the trans-Golgi. The cisternae of the trans-Golgi are clearly associated with the actual calcification of the organic baseplates that subtend the mature coccoliths. We anticipate that several polypeptides implicated with the regulation of the calcification event are localized on those membranes. Thorough characterization under appropriate physiological conditions will suggest which of these polypeptides will be isolated and intensively studied. Following isolation we would be in a position to generate monospecific antibodies and use them for screening the cDNA library.

INVENTIONS (YEAR 1): none

PUBLICATIONS AND REPORTS (YEAR 1):

1. No reports have been published so far.
2. This annual report will be distributed to the ONR Distribution list.

TRAINING ACTIVITIES: One graduate student and two undergraduates have contributed to this project so far.

Women or minorities - 3
Non-citizens - 0

AWARDS AND FELLOWSHIPS: None

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DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By _____	
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Availability Codes	
Dist	Avail and/or Special
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10711
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